Local Land-Use Impact on the Isotopic Character and
Age of Carbon in Unglaciated Small Temperate Watersheds

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By

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Abstract

The impact of local land-use practices on the transfer of carbon from land to small headwater streams is understudied despite the fact that it has the most pronounced impact on stream carbon at these scales. We measured the stream water concentration, $\delta^{13}C$, and $\Delta^{14}C$ of dissolved organic carbon [DOC], particulate organic carbon [POC], and dissolved inorganic carbon [DIC] in temperate headwater streams. The streams drained a forested, unimproved pasture, large and small mixed land-use, and tilled and no-till corn watershed in Coshocton, Ohio in the fall, spring, and summer and during a winter and spring storm event. Annual carbon fluxes of DOC and POC were greatest in the tilled corn watershed than in all other watershed types. Stream $\delta^{13}C$-DOC values indicate that this carbon pool was mainly derived from the present overlying vegetation. $\Delta^{14}C$-DOC was all modern suggesting vegetative root exudates contributing to the DOC pool. $\Delta^{13}C$-POC values indicate that this carbon pool was derived from a mixture of C_3 vegetation and soil organic matter in the non-corn watersheds, and C_4 vegetation in both corn watersheds. $\Delta^{14}C$-POC values indicate that in the non-corn watersheds, this carbon pool was derived from a mixture of modern overlying vegetation and deeper pre-agricultural soil. Corn $\delta^{13}C$-DIC and $\Delta^{14}C$-DIC indicate that DIC was derived from atmospheric CO_2 equilibration interactions suggesting that respired DOC and POC minimally contribute to the DIC pool. $\delta^{13}C$-DIC and $\Delta^{14}C$-DIC values in the non-corn watersheds indicate that a mixture of carbonate bedrock dissolution and respiration of organic matter are the primary sources of carbon to this pool. Overall, the flux, isotopic signature, and radiocarbon age of the three carbon pools show that crop practices, especially when tilled, enhance carbon delivery to streams and reduce the residence time of carbon in the watershed.

Introduction

Shifts in land-use can affect the production, respiration, and transportation of carbon in watersheds. Siemens *[2003]* hypothesized that the leaching of dissolved inorganic and organic carbon from soils can explain a large part of the difference between atmosphere- and land-based estimates of the carbon uptake of terrestrial ecosystems. Similarly, Cole et al. *[2007]*, and Battin et al. *[2009]* argued that consideration of inland waters as components of terrestrial carbon budgets is necessary to assess the carbon cycle at the landscape scale. Riverine carbon fluxes are very sensitive to regional and global change due to fact that freshwater fluxes reflect physical, biotic and anthropogenic processes [*Raymond et al.,* 2008; *White and Blum,* 1995].

It is known that dramatic shifts in land use due to anthropogenic influences impact the concentration, character, and age of carbon from land to large temperate rivers [*Raymond et al*., 2004] and from tropical rivers and their headwaters [*Townsend-Small et al.,* 2007; *Johnson et al*., 2006; *Moyer et al.,* in review]. However, the sources and fluxes of carbon in small temperate headwater streams from which the larger rivers are derived are not well understood. Far from insignificant, headwater streams are the smallest streams within a watershed and can account for more than 90% of the streams and almost half of all river miles in the United States [*Leopold et al.,* 1964].

Reliable estimates of net watershed carbon balances are therefore of fundamental importance. Only with specific local data, can larger scale studies be adequately interpreted to determine the effect of land use change on the carbon cycle at larger scales. Therefore, this project was designed to determine how specific land use practices affect the dissolved organic carbon (DOC), particulate organic carbon (POC), and dissolved inorganic carbon (DIC) annual fluxes, isotopic character, and age in small temperate headwater streams in Coshocton, Ohio's North Appalachian Experimental Watershed.

Methods

Field Sites

The experiment was conducted at the North Appalachian Experimental Watershed (NAEW) near Coshocton, Ohio (40° 21' N, 81° 46' W) (Fig 1). The NAEW is a 424 ha outdoor laboratory facility maintained by the USDA-Agricultural Research Service and provides a 70-year monitoring database of each of the studied watersheds including crop rotation and water flow records. The NAEW is representative of the climate, land management, vegetation cover and geologic composition of the temperate hill region of southeastern Ohio, western Pennsylvania, and parts of West Virginia, Kentucky, Tennessee, and southern Indiana [*Owens et al.*, 2008]. Natural vegetation at the NAEW has been classified as mixed oak forest with white oak (*Quercus alba*), black oak (*Q. velutina*) and hickory (*Carya* spp.) being predominant species [Izaurralde et al., 2007]. The NAEW is at 300 to 600 m above sea level, and does not receive runoff water from surrounding areas. Annual long-term precipitation averages 973 mm. Maximum daily air temperature averages 16.0 °C and minimum daily air temperature averages 3.4 °C [*Kelley et al.*, 1975]. The soils at NAEW are non-glaciated and developed from residuum and colluvium parent materials derived from sedimentary bedrock such as coarse-grain sandstone, shale, and limestone [*Kelley et al.*, 1975].

Water samples were collected from six watersheds (WS): 1) a forested watershed (F, WS #172, 17.7 ha) that is 100% undisturbed forested land, 2) an unimproved pasture watershed (UP, WS #182, 28.8 ha) consisting of 82% pasture and 18% forested, 3) a large mixed land-use watershed (LMU, WS #196, 122.7 ha) that is 43% meadow, 34% wooded, 14% high fertility pasture, and 9% fertilized cropland, 4) a small mixed land-use watershed (SMU, WS #166, 32.1 ha) that is a mixture of 34% meadow, 32% fertilized cropland, 23% “medium fertility” pasture, 13% summer pasture, 11% wooded, and 10% year round pasture for feeding, 5) a no-till corn watershed (NT, WS #115, 0.65 ha) and 6) a disk-tilled corn watershed (T, WS #127, 0.67 ha) (Table 1) (Fig 1) [*Owens et al.*, 2008; *Shipitalo and Owens et al.*, 2006]. The disk-tilled watershed was tilled to a depth of approximately 10 cm three to four times before planting each year [*Owens et al.*, 2008 and *Shipitalo and Owens et al.*, 2006]. The land management rotation and land applications histories were provided by the NAEW from 1939 to 2009 for all studied watersheds (Tables 2- 4).

Experimental Design

The headwater streams flowing through the forested, unimproved pasture, large mixed land-use and small mixed land-use watersheds were sampled seasonally on one base flow day in the fall (25 October 2008), winter (9 February 2009), spring (25 April 2009), and summer (18 August 2009). In addition, the streams in all four sites, as well as the runoff from the tilled corn and no-till corn watersheds, were sampled during a high flow winter snow-melt (9 February 2009) and a spring storm event (2 May 2009).

Each of the non-corn watersheds is equipped with a 2:1 broad-crested weir to measure stream flow [*Owens et al*., 2008]. The tilled and no-till corn watersheds have ephemeral streams such that water was collected during a high flow events from a rotating vane sampler in a gauge shed onsite and kept frozen until analyzed. A preferred collection site (i.e., above the weir) and a secondary collection site (i.e., below the weir), was designated along the stream within each non-corn watershed. Water samples were always taken from the preferred collection site above the weir unless flow was too low and then the water samples were taken from below the weir (Table 5).

Collection Methods and Analysis

Water was collected for concentration, $\delta^{13}C$, and $\Delta^{14}C$ of DOC, POC, and DIC (Table 5). A thorough water sample collection and analysis method is described in *Raymond and Bauer* [2001b]. In summary, stream water was filtered through a pre-baked (500 °C) Whatman QMA quartz fiber filters (0.7 µm nominal pore size) with a peristaltic pump equipped through a 10% HCl rinsed silicone tubing and filter housing. Filtered DOC samples were collected in pre-cleaned (10% HCl) polycarbonate bottles and both were kept on ice in the field and immediately transferred to a -20 °C freezer upon return to the lab. The filters containing the POC samples were individually stored in pre-baked (500 °C) aluminum foil, sealed in a plastic bag, and stored frozen at -20 °C. DIC samples were stored in pre-cleaned (10% HCl, baked at 500 °C) crimp sealed glass serum bottles and fixed with mercuric chloride. DIC samples were kept in the dark and stored at room temperature. Latitude, longitude, salinity, pH, water temperature, and the volume of water passed through each filter were recorded at each sampling site each season (Table 6).

$\delta^{13}C$ and concentration analyses

The frozen DOC samples were sent to the National Ocean Sciences Accelerator Mass Spectrometry facility (NOSAMS) at Woods Hole Oceanographic Institute and analyzed for $\delta^{13}C$ in their Organic Mass Spectrometry Facility. A subsample of each collected water sample was combusted in a Carlo Erba/Fisons 1108 Elemental Analyzer at 1700 °C within an oxygen rich gas stream in the combustion tube. The resulting CO_2 gas was transferred via a Conflo II system to a Finnigan-MAT Delta^{plus} IRMS and the $\delta^{13}C$ and concentration of the DOC measured. $\delta^{13}C$ was reported as the per mil deviation of the ratio of ^{13}C: ^{12}C relative to Vienna Pee Dee belemnite (VPDB) limestone standard **[***Coplen*, 1996]. The remainder of each water sample was UV-oxidized and the resulting CO_2 cryogenically purified, according to methods described in Beaupre et al. **[***2007*] in preparation for $\Delta^{14}C$ analysis (see $\Delta^{14}C$ analysis details below).

POC filters were dried and HCl-fumed order to remove any inorganic carbon in the samples [*Lorrain et al*., 2003], then combusted in a Costech Elemental Analyzer and the POC concentration measured. The resulting CO_2 gas was automatically transferred via a Finnigan ConFlow III open split interface to a Finnigan Delta IV Plus IRMS for the analysis of the $\delta^{13}C$-POC and reported relative to VPDB in Grottoli's Stable Isotope Biogeochemistry Laboratory at Ohio State University (SIBLab-OSU). At least fourteen percent of all samples were analyzed in duplicate.

DIC samples were prepared for isotopic analyses using methods based on those of *Raymond and Bauer* [2001b].Briefly, each sample was acidified with 85% orthophosphoric acid, sparged under ultra-high purity (UHP) helium flow, the resulting CO_2 gas was cryogenically purified under vacuum, and the DIC concentrations were obtained from the pressure-volume relationship of a calibrated section of the vacuum line. The resultant CO_2 gas was then split into two glass ampoules: one for $\delta^{13}C$ and one for $\Delta^{14}C$ analyses. One ampoule was cracked into a Finnigan Delta IV Plus IRMS via an automated multi-port system and the $\delta^{13}C$-DIC measured and reported relative to VPDB in Grottoli's SIBLab-OSU. Repeated measurements of an internal standard (n_{DIC} = 13) had a standard deviation of $\leq\pm0.02$‰. At least fourteen percent of all samples were analyzed in duplicate.

$\Delta^{14}C$ Analyses

The $\Delta^{14}C$ of DOC, POC, and DIC samples were analyzed by National Ocean Sciences Accelerator for Mass Spectrometry (NOSAMS) Facility at Woods Hole Oceanographic Institute. For all samples, CO_2 gas was reduced to graphite using H_2 gas and Fe as a catalyst and the ratio of $^{14}C/^{12}C$ was measured via Accelerator Mass Spectrometry (AMS), blank-subtracted for background correction, and further corrected for fractionation using $\delta^{13}C$ values measured by IRMS in the Stable Isotope Biogeochemistry Lab at the Ohio State University. All radiocarbon values were reported by the AMS laboratories as fraction modern and converted to $\Delta^{14}C$ (the per mil deviation of ^{14}C:^{12}C relative to the 95% oxalic acid-1 standard) and ^{14}C age (*Stuiver and Polach*, 1977).

Annual Flux Calculations

Flow data of each stream was obtained from the NAEW archive. All measurements were averaged per day and converted to m^3 day^{-1}. The number of days per season (Fall, Winter, Spring, and Summer) as well as the number of base flow and storm flow days was counted (Table 6). Days were determined to be storm flow days when water drained from the ephemeral streams of the corn watersheds. The total number of base flow and storm flow days was then multiplied by the average base flow and storm flow rates per season, respectfully, and the total volume of water discharged per season was calculated. For each carbon pool (DOC, POC, DIC), the total water flow per season was then multiplied by the concentration (kg-C) to get the seasonal fluxes. The seasonal fluxes for each carbon pool were then added up to get a total annual flux for each watershed, and normalized to watershed area (km^2) yielding the annual carbon flux per watershed values for each carbon pool.

Data analysis

A fully factorial model III analysis of variance (ANOVA) was used to test for differences between watersheds and seasons for each of the following variables: [DOC], $\delta^{13}C$-DOC and $\Delta^{14}C$-DOC, [POC], $\delta^{13}C$-POC and $\Delta^{14}C$-POC, and [DIC], $\delta^{13}C$ -DIC and $\Delta^{14}C$-DIC. Duplicate values were averaged prior to ANOVA analysis to avoid pseudo-replication errors. Averaged values were reported as arithmetic means ±1 standard deviation, and differences were considered statistically significant at $p \leq 0.05$. All statistical analyses were conducted using SAS version 9.1.3 of the SAS System for Windows (© 2000-2011 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, North Carolina, USA.). All graphs were created with SigmaPlot version 10.0 for Windows (© 2006 Systat Software, Inc.). The ranges of $\delta^{13}C$ and $\Delta^{14}C$ values for the most likely possible sources of carbon to the watersheds were obtained from Moyer *et al.* [in review] and plotted relative to the measured DOC, POC, and DIC $\delta^{13}C$ and $\Delta^{14}C$ values. The possible contribution of the various carbon sources to each carbon pool was visually assessed.

Results

Carbon Fluxes

Total annual DOC and POC fluxes were highest in the no-till and tilled corn watersheds compared to all other watershed types even though the corn site fluxes were entirely derived from storm flow event (Fig 2A, B). The small mixed land-use, large mixed land-use, unimproved pasture, and forested watersheds base flow and storm flow DOC and POC fluxes were similar and contributed nearly equally to the total annual fluxes of these carbon pools (Fig. 2A, B).

The total annual DIC flux was highest in the tilled corn, followed by the forested watershed (Fig. 2C). Total annual DIC fluxes were similar in all other watersheds (Fig. 2C). Among the non-corn watersheds, DIC base flow fluxes were similar and storm flow DIC flux was highest in the forested watershed (Fig. 2C).

$\delta^{13}C$ and $\Delta^{14}C$ Isotopes

Stream δ^{13}C-DOC was significantly enriched in both the no-till and tilled corn watersheds compared to the remaining watersheds (Table 8; Fig. 3A). The Δ^{14}C-DOC did not significantly differ between watersheds and were all modern (Table 8; Fig. 3A). δ^{13}C-DOC and Δ^{14}C-DOC was not statistically different among seasons (Table 8).

Stream δ^{13}C-POC was significantly enriched in both no-till and tilled corn watersheds compared to the remaining watersheds (Fig.3B). Though not statistically testable, the fall small mixed land-use δ^{13}C-POC value was noticeably more depleted in comparison to all other δ^{13}C-POC values. Average Δ^{14}C-POC of both corn watersheds was younger than the average Δ^{14}C-POC of all other watersheds (Table 8; Fig. 3B). The Δ^{14}C-POC of the non-corn watersheds ranged from modern to slightly aged. However, δ^{13}C-POC and Δ^{14}C-POC did not statistically differ among seasons (Table 8).

Stream δ^{13}C-DIC was significantly enriched in both no-till and tilled corn watersheds compared to the remaining watersheds (Table 8; Fig. 3C). Δ^{14}C-DIC was youngest in the corn watersheds and ranged from modern to slightly aged in all other watersheds (Fig. 3C). Overall, δ^{13}C-DIC and Δ^{14}C-DIC did not statistically differ among seasons (Table 8).

Discussion

The observed differences in the concentrations, fluxes, and isotope data for each the carbon pools in the six watersheds in the NAEW (Tables 5-8; Figs. 2 & 3) are likely being driven by a complex set of interactions between biological, chemical and geological processes. The identification of the sources of carbon to rivers has helped constrain the fluxes of carbon in temperate [*Masiello and Druffel,* 2001; *Raymond et al*., 2004] and tropical river systems [*Moyer et al*., in review]. δ^{13}C-DOC (Fig. 3A), δ^{13}C-POC (Fig. 3B), and Δ^{14}C-DIC (Fig. 3C) are likely source-driven, and an explanation of those carbon sources to the temperate watershed stream system is necessary to understand the fluxes of DOC, POC, and DIC with respect to changing land-use. Therefore, the results are interpreted here in terms of identifying sources of organic and inorganic carbon to streams, explaining the observed statistical patterns in the data, and constraining the calculated fluxes of carbon to temperate headwater streams.

Sources of organic carbon (DOC and POC) to temperate headwater streams

Overall, storm events were responsible for 50-100% of the total annual DOC and POC stream export, even though storm events only occurred on ~32% of the days during the 2008-2009 sampling period. In addition, the no-till corn and tilled corn watersheds had much higher fluxes of total DOC and POC per year than the non-corn watersheds, even though the corn watershed streams only flowed during storm events (Fig. 2A, B). Therefore, the cropland management practices are increasing carbon movement and decreasing the quality and quantity of soil organic matter [*Dalal et al.,* 2011; *Wilman et al.,* 2011].

Young stream Δ^{14}C-DOC across all watersheds suggests that recently deposited POC is microbially reactive and the largest contributor to stream DOC (Fig. 3A). In addition, young stream Δ^{14}C-POC in both corn watersheds indicates that modern vegetation litter is the primary source of POC (Fig. 3B). This indicates that recently produced organic matter from contemporary vegetation is the primary source of carbon released from croplands. In contrast, this differs from Northeast United States temperate rivers (NE US temperate rivers) and tropical small mountainous (SMRs) which are primarily depleted Δ^{14}C-POC from cropland use [*Raymond et al.,* 2004]. Thus, the influence of cropland production intensifies the fluxes of young and δ^{13}C-enriched DOC and POC to move through small temperate watersheds quickly compared to the non-corn watersheds.

In addition, one δ^{13}C-POC data point was anomalous but could suggest that algal biomass and/or an unconstrained source can contribute to the POC pool during the lack of flow during the fall period. However, equally depleted δ^{13}C-POC has been reported for [*France and Cattaneo,* 1998]. In contrast, previous studies in larger North East United States temperate rivers (NE US temperate rivers) indicate Δ^{14}C-DOC and δ^{13}C-DOC is derived from a mixture of older pre-agricultural soil and young C_3 vegetation, while SMRs reveal older and a wider range of δ^{13}C-DOC compared to the younger, C_3-constrained δ^{13}C-DOC exported from non-corn temperate headwater streams in this study [*Raymond et al.,* 2004; *Loh et al.,* 2006; *Moyer et al*., in review].

However, the δ^{13}C character and age of the POC in the non-corn watersheds varies due to the varying contribution of modern overlying C_3 vegetation and pre-agricultural soil pools (Fig. 3B). In comparison, the temperate headwater streams in this study are more similar to values of SMRs and encompass a larger range of ages than the δ^{13}C values reported for NE US temperate rivers [*Raymond and Bauer,* 2001a; *Raymond et al.,* 2004; *Loh et al.,* 2006, *Moyer et al.,* in review]. Fractionation of δ^{13}C-POC may be due to preferential substrate utilization, which is the utilization and decomposition of preferred substrates of carbon [*Werth and Kuzyakov,* 2010]. This suggests that DOC becomes more aged as you move downstream from temperate headwater streams and into the main river stems due to longer residence times and erosion of organic rich sedimentary bedrock within the river system.

The fluxes of DOC and POC indicate that the conversion of land to crop production increases the export of modern vegetation, while non-corn watersheds export a mixture of vegetation and pre-agricultural soil of these carbon pools. The older organic carbon that was found in larger temperate watersheds does not reveal the incorporation of younger organic carbon that is exported from crop production in these small temperate watersheds [*Raymond et al.,* 2004]. As a result, the findings of these small temperate watersheds provide an additional understanding of the impact of land use to a specific region in relation to the broader river system of carbon cycling.

Sources of dissolved inorganic carbon to temperate headwater streams

All of the watersheds export the most carbon in the form of DIC (Fig. 2C). The tilled corn watershed exported the most DIC per unit area, followed by the forested watersheds (Fig. 2C). The primary sources of DIC in most river systems is the oxidation of organic matter via both abiotic (e.g. photo-oxidation) and microbial process (e.g. soil and aquatic respiration), and the weathering of carbonate and siliciclastic rocks [*Telmer and Veizer*, 1999] or lime as applied to cropland to buffer the soil (Table 4). All three sources are possible in the NAEW. In the NAEW, the soils are non-glaciated and developed from residuum and colluvium parent material derived from sedimentary bedrock such as coarse-grain sandstone, shale, and limestone [*Kelley et al.*, 1975]. In addition, crushed limestone consisting of mainly calcite and some dolomite was applied to some watersheds over their history (Table 4). A sample of the crushed limestone was obtained from NAEW and plotted in Figure 3C with an asterisk.

In the forested watershed, high fluxes of aged DIC coupled with low DOC and POC and no history of limestone applications in that watershed indicates that carbonate weathering is most likely the primary carbon source to the DIC pool (Figs 2C, 3A-C). Further study is necessary to determine if the underlying bedrock of the NAEW is heterogeneous and/or if the conditions in the forested watershed are conducive to higher carbonate weathering rates than the other watersheds. In the case of both corn watersheds and the small mixed land-use, crushed limestone was applied as a soil buffer (Table 4). However, relatively large DIC fluxes were only observed in the tilled corn site. This would suggest that by increasing the surface area via tilling, limestone dissolution is enhanced and the total net flux of DIC is greater than in comparable non-tilled watersheds.

The character and age of DIC moving through temperate headwater streams differ from the ranges reported for SMRs. SMRs have similar δ^{13}C-DIC ranges but are more modern compared to the DIC exported from non-corn temperate headwater streams that indicating higher respiration rates with warmer climates [*Townsend-Small et al.*, 2007; *Moyer et al.*, in review]. There is more similarity among δ^{13}C-DIC between temperate headwater streams and NE US temperate rivers indicating a mixture of young organic matter respiration and weathering of bedrock [*Raymond et al.*, 2004]. However, temperate headwater streams are still more aged than the NE US temperate rivers suggesting that a significant source of DIC to small non-glaciated temperate headwater streams is bedrock.

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Table 1. Watershed sizes, cropping management, and fertilizer treatment.

WS Name	WS #	Area (ha)*	Cropping Management *	Fertilizer Treatment *
Tilled Corn	127	0.67 †	See Table 3	Slope: 9% †
No-Till Corn	115	0.65 †	See Table 3	Slope: 7% †
Forested	172	17.7	All wooded (100%)	No chemical treatment
Unimproved Pasture	182	28.8	23.6 ha unimproved pasture (82%) 5.2 ha wooded (18%)	No chemical treatment No chemical treatment
Small Mixed Land-Use	166	32.1	10.8 ha meadow (34%) 7.4 ha "medium fertility" pasture (23%)	No chemical treatment
			4.3 ha summer pasture (13%) WS 135 (Separate WS within WS 166)	56 kg N ha^{-1} y^{-1} (3 years); 168 kg N ha^{-1} y^{-1} (11 years); 0 kg N ha^{-1} y^{-1} (11 years)
			3.1 ha year round pasture/feeding (10%) WS 129 (Separate WS within WS 166)	300 kg N ha^{-1} y^{-1} from hay WS 129 (Owens et al. 1982) (12 years); 0 kg N ha^{-1} y^{-1} (13 years)
			10.3 ha fertilized cropland (32%) 3.6 ha wooded (11%)	
Large Mixed Land-Use	196	122.7	52.5 ha meadow (43%) 41.7 ha wooded (34%) 17.1 ha high fertility pasture (14%) WS 110 (summer rotation; Separate WS within WS 196); WS 106 (winter rotation; Separate WS within WS 196) 11.4 ha fertilized cropland (9%)	No chemical treatment No chemical treatment 224 kg N ha^{-1} y^{-1} (4 years); Legume-grass mixture, 0 kg N ha^{-1} y^{-1} (21 years)

* Owens et al., 2008; † Shipitalo and Owens et al., 2006

Table 2. Watershed cropping management rotation history (1939-2009). The annual cycle ran May through April of each year.

Year	Forested (WS 172)	Unimproved Pasture (WS 182)	Small Mixed Land-Use (WS 166)	Large Mixed Land-Use (WS 196)
1939-1970	Forested	-	-	-
1971-1973	Forested	-	Disked, seeded grass, applied lime, planted corn, fertilized, herbicide, mowed/raked/baled hay	Fertilized grass and corn
1974	Forested	-	Cows fed hay and worked	-
1975	Forested	Mowed/raked/baled hay, bush hogged	Planted corn, fertilized, herbicide, mowed/raked/baled hay and alfalfa	-
1976	Forested	Mowed/raked/baled hay, bush hogged pasture	Cows fed hay and worked	-
1977	Forested	Cows fed hay and worked, mowed/raked/baled hay, bush hogged pasture	Cows fed hay and worked, planted corn, fertilized, herbicide, mowed/raked/baled hay	-
1978	Forested	Cows fed hay and worked, mowed/raked/baled hay, bush hogged pasture	Cows fed hay and worked, disk tilled oats, hay, pasture, fertilized, herbicide, mowed/raked/baled hay, bush hogged pasture	-
1979-1984	Forested	Cows fed hay and worked, mowed/raked/baled hay, bush hogged pasture	Cows fed hay and worked, fertilized, herbicide, mowed/raked/baled hay	-
1985-2006	Forested	Cows fed hay and worked, mowed/raked/baled hay, bush hogged pasture	Cows fed hay and worked, fertilized, herbicide, plant/harvest: orchard, alfalfa, rye, red clover, straw, hay, silage, hay, rotary hoe tilled corn	-
2007	Forested	Cows fed hay and worked	Cows fed hay and worked, mowed/raked/baled hay	Cows fed hay and worked, disk tilled corn
2008	Forested	Cows fed hay and worked	Cows fed hay and worked, fertilized, mowed/raked/baled hay	Cows fed hay and worked, fertilized, disk tilled corn
2009	Forested	Cows fed hay and worked	Cows fed hay and worked, mowed/raked/baled hay	Cows fed hay and worked, fertilized

All data provided by the NAEW

Table 3. Agricultural watershed cropping management rotation history (1939-2009). The annual cycle ran May through April of each year.

Year	Tilled Corn (WS 127)	No-Till Corn (WS 115)
1939	-	Oats
1940-1946	-	Wheat/Meadow/Corn
1947-1969	Plow-Plant Corn/Wheat/Meadow	Corn/Wheat/Meadow
1970	Meadow	Soybean Study Strips
1971	Meadow	No-Till Corn
1972	Meadow	No-Till Corn
1973-1974	No-Till Corn	No-Till Corn
1975-1977	Meadow	Meadow
1978-1982	No-Till Corn	No-Till Corn
1983	Wheat, Red Clover, Plant Rye	Wheat, Red Clover, Plant Rye
1984-1989	Paraplow Corn/Paraplow Soybean	Paraplow Corn/Paraplow Soybean
1990	Wheat, Meadow Strips	Disk tilled Soybean
1991	Disk tilled Corn	Wheat, Meadow Strips
1992	Disk tilled Soybean	Disk tilled Corn
1993	Wheat, Red Clover	Disk tilled Soybean
1994	Disk tilled Corn	Wheat, Red Clover
1995	Disk tilled Soybean	Disk tilled Corn
1996	Wheat, Red Clover	Disk tilled Soybean
1997	Disk tilled Corn	Wheat, Red Clover
1998	Disk tilled Soybean	Disk tilled Corn
1999	Wheat, Red Clover	Paraplow/disk tilled Soybean
2000	Paraplow/disk tilled Corn	Wheat, Red Clover
2001	Paraplow/disk tilled Soybean	Paraplow/disk tilled Corn
2002	Wheat, Red Clover	Paraplow/disk tilled Soybean
2003	Paraplow/disk tilled Corn	Wheat, Red Clover
2004	Paraplow/disk tilled Soybean	Paraplow/disk tilled Corn
2005	Wheat, Red Clover	Paraplow/disk tilled Soybean
2006	Paraplow/disk tilled Corn	Wheat, Red Clover
2007	Paraplow/disk tilled Corn	Paraplow/disk tilled Corn
2008	Paraplow/disk tilled Corn	No-Till Corn
2009	Paraplow/disk tilled Corn	No-Till Corn

Wheat, Red Clover: Winter wheat over-seeded with red clover in the same season

All data provided by the NAEW

Table 4. Application schedules of fertilizer, manure, and lime to each watershed.

WS Name	WS #	Year	Total N (kg/ha)	Total P (kg/ha)	Total K (kg/ha)	Total Manure (loads)	Total Lime (kg/ha)
Tilled Corn	127	1947-1979	454	1767	1767	40	17297
		1980-1999	490	490	992	36	7413
		2000-2009	714	528	376	-	6178
		Total Applied	1658	2785	3135	*76*	30888
No-Till Corn	115	1938-1979	985	774	732	-	16062
		1980-1999	1415	986	1994	40	12355
		2000-2009	556	367	342	12	6178
		Total Applied	*2956*	*2127*	*3068*	*62*	*34595*
Forested	172	1939-2009	-	-	-	-	-
Unimproved Pasture	182	1963-2009	-	-	-	-	-
Small Mixed Land-Use	166	1966-1979	1311	507	1026	-	2471
		1980-1999	4991	1740	5137	-	24711
		2000-2009	1195	16	905	-	-
		Total Applied	*7497*	*2263*	*7068*	-	*27182*
Large Mixed Land-Use	196	1936-1979	1113	32	32	-	-
		1980-1999	-	-	-	-	-
		2000-2009	874	227	120	-	-
		Total Applied	*1987*	*259*	*152*	-	-

All data provided by the NAEW

Table 5. Stream sampling location in each watershed and season. WS = watershed, TC = tilled corn, NTC = no-till corn, F = forested, UP = unimproved pasture, SMU = small mixed land use, LMU = Large mixed land use

Season	TC	NTC	F	UP	SMU	LMU
Fall 08	-	-	Above weir	Above weir	Below weir	Below weir
Winter 09	Gauge shed	Gauge shed	Below weir	Above weir	Above weir	Below weir
Spring 09	-	-	Above weir	Above weir	Above weir	Below weir
Spring Storm 09	Gauge shed	Gauge shed	-	Above weir	Above weir	-
Summer 09	-	-	Below weir	-	Below weir	Below weir

Table 6. Watershed Dissolved Oxygen (DO, mg/L) and Temperature (°C) by season. WS = watershed, TC = tilled corn, NTC = no-till corn, F = forested, UP = unimproved pasture, SMU = small mixed land use, LMU = Large mixed land use

WS Name	WS #	Fall 08 DO/Temp	Winter SM 09 DO/Temp	Spring 09 DO/Temp	Sp. Storm 09 DO/Temp	Summer 09 DO/Temp
TC	127	N/A	12.71/ 5.6	N/A	10.21/10.8	N/A
NTC	115	N/A	13.64/5.6	N/A	10.20/10.9	N/A
F	172	8.20*/10.5*	12.96/2.9	9.00/17.7	N/A	2.09*/22.3*
UP	182	6.85/12.6	13.25/3.4	9.75/16.8	9.65/15.9	N/A
SMU	166	8.96*/9.0*	13.17/1.6	8.34/17.6	9.06/14.5	2.1*/21.4*
LMU	196	9.92/12.2	14.48/2.4	11.23/15.7	N/A	4.72/27.4

*Sample taken from secondary site

Table 7. Number of base flow and storm flow days during the experimental sampling period.

Season	Number of Base Flow Days	Number of Storm Flow Days
Fall 08	82	8
Winter 09	70	19
Spring 09	81	12
Summer 09	86	7
Total Days	*319*	*46*

Table 8. Results of a fully factorial model III analysis of variance (ANOVA) for both δ^{13}C- DOC, POC, and DIC and Δ^{14}C-DOC, POC, and DIC. df = degrees of freedom. SS = sum of squares. F = calculated F statistic. p = probability. Effect is significant when $p \leq 0.05$.

δ^{13}C-DOC	Df	SS	F	P	Δ^{14}C-DOC	df	SS	F	p
Model	9	334.14	5.73	0.0079	Model	9	6659.49	1.85	0.1858
Watershed	5	231.41	7.14	0.0058	Watershed	5	3583.96	1.80	0.2099
Season	4	69.10	2.67	0.1021	Season	4	553.47	1.39	0.3131
δ^{13}C-POC	Df	SS	F	P	Δ^{14}C-POC	df	SS	F	p
Model	9	620.67	10.67	<0.001	Model	9	26211.42	3.98	0.0258
Watershed	5	263.40	8.15	<0.005	Watershed	5	16977.91	4.64	0.0226
Season	4	82.07	3.17	0.0693	Season	4	660.26	0.23	0.9172
δ^{13}C-DIC	Df	SS	F	P	Δ^{14}C-DIC	df	SS	F	p
Model	9	152.20	9.83	<0.001	Model	9	74760.15	3.23	<0.005
Watershed	5	60.53	7.03	0.0061	Watershed	5	40130.34	3.12	0.0660
Season	4	9.27	1.35	0.3251	Season	4	5250.09	0.51	0.7303

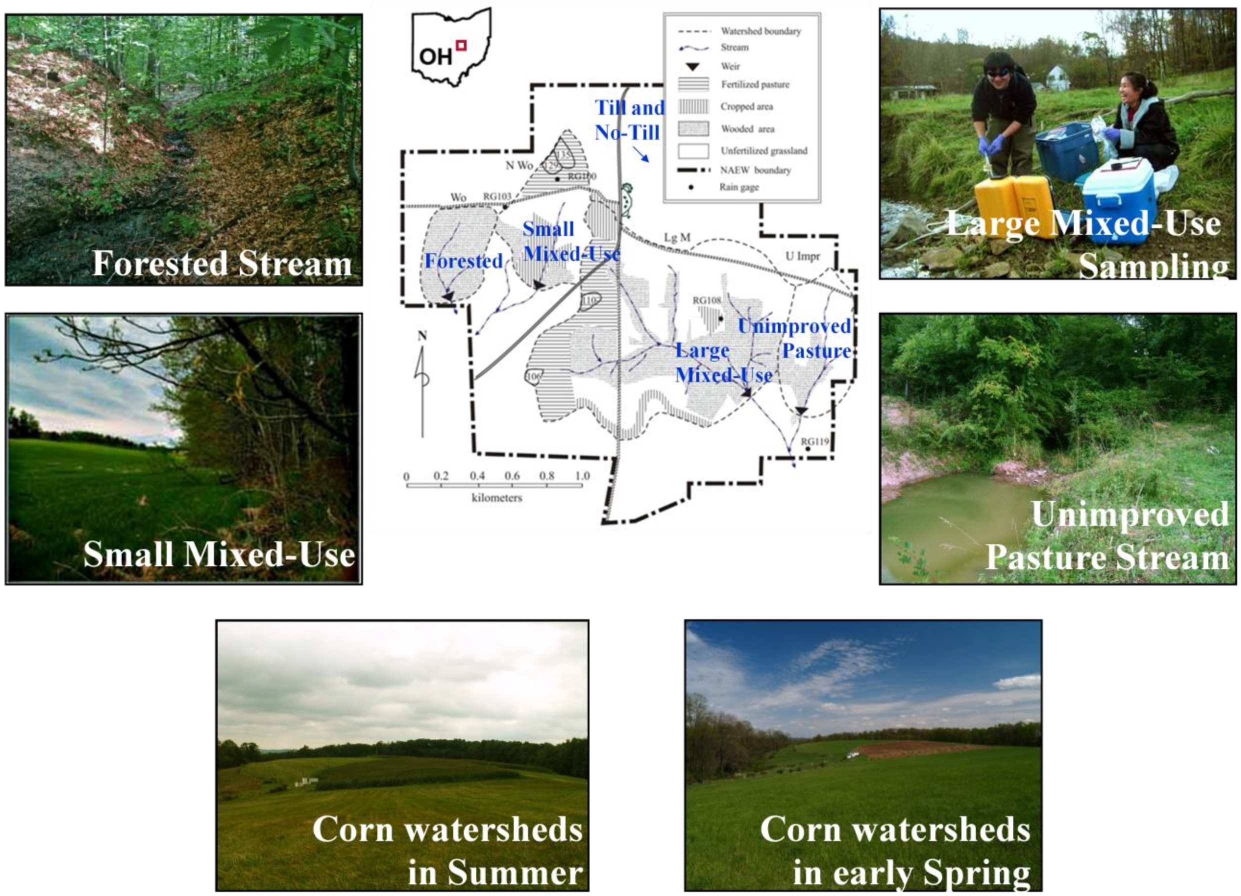

Figure 1. The location of the studied watersheds within the North Appalachian Experimental Watershed (NAEW) [*Owens et al.,* 2008]. The small set map indicates the NAEW's location in Coshocton, Ohio, USA. The photographs show watershed conditions during various sampling days.

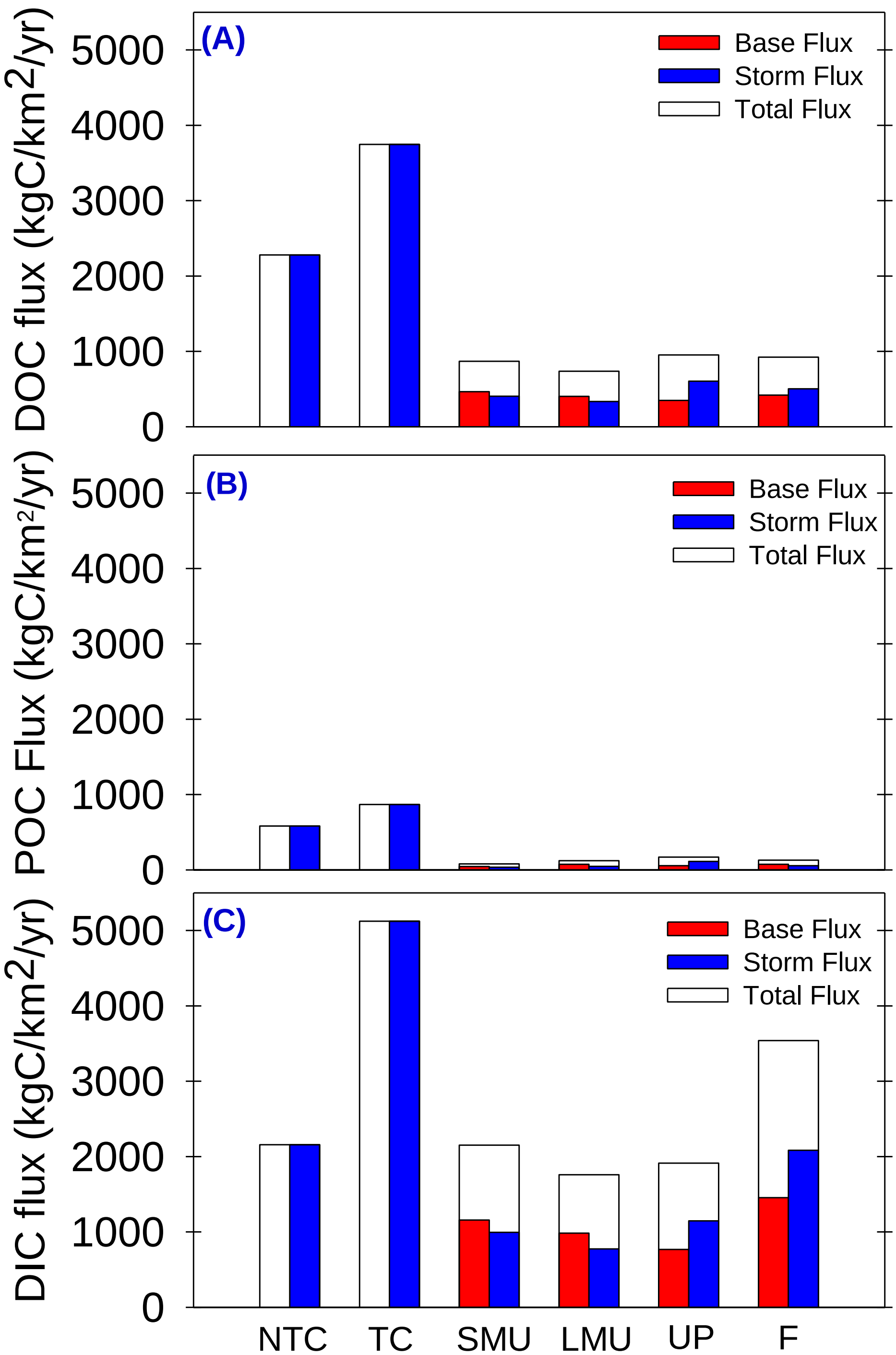

Figure 2. Annual carbon fluxes for A) dissolved organic carbon (DOC), B) particulate organic carbon (POC), and C) dissolved inorganic carbon (DIC) for all watersheds. The watersheds are no-till corn (NTC), tilled corn (TC), small mixed land-use (SMU), large mixed land-use (LMU), unimproved pasture (UP), and forested (F). Total annual fluxes are the sum of the average annual base flux plus the average storm flux per watershed.

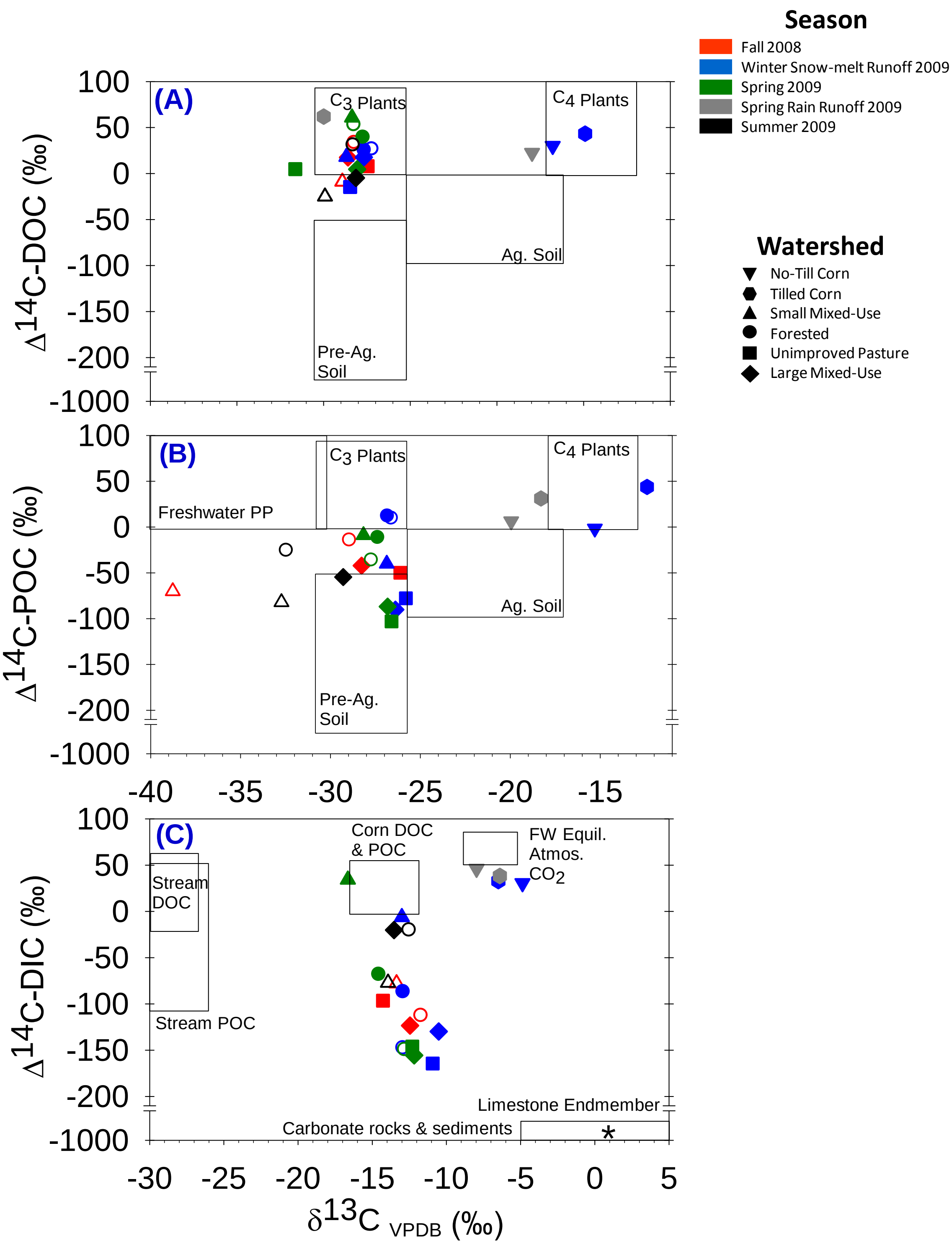

Figure 3. $\Delta^{14}C$ vs. $\delta^{13}C$ plots of A) dissolved organic carbon (DOC), B) particulate organic carbon (POC), and C) dissolved inorganic carbon (DIC) values from each studied watersheds. In C), stream DOC and POC boxes are defined by the range of measured values in A) and B). The ranges of published values for all other possible sources of carbon are represented by the reference boxes (values from *Moyer et al.*, in review). Freshwater primary production (Freshwater PP), pre-agricultural soil (Pre-Ag. Soil), agricultural soil (Ag. Soil), freshwater equilibrium atmospheric CO_2 (FW Equil. Atmos. CO_2).